

# BIOHYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT VIA SEQUENTIAL DARK-PHOTO FERMENTATION

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## **SUPERVISOR'S DECLARATION**

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

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## **STUDENT'S DECLARATION**

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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Thesis submitted in fulfillment of the requirements  
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## **DEDICATION**

“Specially dedicated to my beloved grandfather Late Shri Gangadhar Mishra, my parents and my lovable sisters Priyanka and Monika Mishra, who constantly encouraged supported me all the way since the beginning of the studies”.

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## ABSTRAK

Penghasilan hidrogen secara fermentasi daripada biomass (iaitu produk yang terhasil melalui fotosintesis) menjanjikan peluang ke arah penghasilan tenaga yang lestari. Konsep baru berbekalkan dua peringkat penembusan gelap dan fotografi (TSDPF) telah dicadangkan untuk meningkatkan penghasilan hydrogen ( $H_2$ ) dan  $COD_{\text{removal}}$  menggunakan efluen minyak kelapa sawit (POME) dari kilang sebagai substrat bagi proses fermentasi dalam kajian ini. Objektif utama kajian ini adalah penghasilan  $H_2$  dari POME menggunakan sistem TSDPF melalui penghasilan secara berkelompok. Pada permulaan kajian, pengasingan bakteria dari POME telah dilakukan bagi memperoleh bakteria penghasil  $H_2$  yang dinamakan '*Bacillus* strain PUNAJANI'. Melalui beberapa data analysis parameter fiziko-kimia yang dicadangkan dalam kajian ini, parameter optimum yang diperoleh adalah pada suhu  $35^\circ\text{C}$ , pH 6.5,  $1.2 \text{ g L}^{-1} \text{ NH}_4\text{Cl}$  (sebagai sumber nitrogen) dan  $10 \text{ g L}^{-1}$  mannose (sebagai sumber karbon) untuk pengeluaran bio- $H_2$  maksimum sebanyak  $2.42 \text{ mol } H_2 / \text{mol}$  heksosa. Disamping itu, bakteria PUNAJANI juga menunjukkan penghasilan  $H_2$  yang berkesan pada  $0.23 \text{ L-}H_2/\text{g-CODremoved}$  apabila POME diguanakn sebagai sumber karbon. Selain itu, nanopartikel nikel dan kobalt oksida yang dihasilkan secara 'hidrothermal' telah ditambah kepada POME dengan julat  $0.25$  hingga  $3.0 \text{ mg L}^{-1}$  POME. Keputusan menunjukkan bahawa POME dengan penambahan  $1.5 \text{ mg L}^{-1} \text{ NiO}$  NPs dan  $1.0 \text{ mg L}^{-1} \text{ CoO}$  NPs mempunyai ciri-ciri sebagai pemangkin dan ianya dapat meningkatkan hasil  $H_2$  sebanyak 1.51 dan 1.67 kali ganda, jika dibandingkan dengan kawalan. Tambahan pula, kajian parameter terhadap pengoptimuman foto-fermentasi  $H_2$  dari POME gelap (DPOME) telah dijalankan menggunakan metodologi statistic pengeluaran Box-Behnken. Hasil eksperimen tindak balas Box-Behnken terhadap permukaan telah menunjukkan kesan positif diantara pembolehubah bersandar (seperti kebegantungan pencairan DPOME, pH awal dan rejim agitasi) terhadap pengeluaran foto- $H_2$ . Hasil maksimum  $H_2$  yang diperoleh adalah pada keadaan optimum 40% pencairan DPOME, pH awal 6.0 dan kadar pengadukan sebanyak 140 rev/min. Melalui penggunaan strategi pengoptimuman ini, peningkatan hasil  $H_2$  yang ketara dari 0.79 hingga 3.11 telah dicapai. Peningkatan pengeluaran  $H_2$  dari DPOME di bawah keadaan optimum telah mencapai peningkatan hampir lima kali ganda. Akhirnya, kebarangkalian sistem TSDPF telah berjaya dilaksanakan menggunakan POME sebagai substrat. Penapaian tahap pertama dilakukan dengan menggunakan PUNAJANI yang terisolasi mempunyai hasil  $H_2$  maksimum  $37.11 \text{ mL-}H_2/\text{g-COD}$  dan 41%  $COD_{\text{removal}}$ . Lebih 40% dicairkan DPOME dengan air paip yang disterilkan untuk kegunaan fermentasi tahap kedua (foto-penapaian). Hasil keseluruhan  $H_2$  dari sistem TSDPF meningkat dari 37.11 sehingga ke  $130.89 \text{ mL-}H_2/\text{g-COD}$ , sementara peratusan  $COD_{\text{removal}}$  secara serentak meningkat dari 41 hingga 93%. Peningkatan pengeluaran  $H_2$  ini lebih tinggi daripada fermentasi POME gelap berperingkat tunggal. Hasil ini memberi kesimpulan terhadap keberkesanan penggunaan POME fermentasi gelap ke arah pengeluaran  $H_2$  yang maksimum dan juga pengurangan kepekatan COD.

## ABSTRACT

Fermentative hydrogen production using biomass (a product of photosynthesis) is a promising route toward the sustainable bioenergy production. A novel concept of two stage-sequential dark-photo fermentation (TSDPF) system was proposed for enhanced biohydrogen production and COD<sub>removal</sub> using palm oil mill effluent (POME) as fermentative substrate. The main objective of this study comprises the hydrogen production in batch mode from POME using TSDPF system. In the initial stage of the study, isolation of an indigenous hydrogen producing strain, '*Bacillus* strain PUNAJAN1' was done using POME sludge. The analytical data of various physicochemical parameters indicated the maximum biohydrogen production of 2.42 mol H<sub>2</sub>/mol hexose at optimal temperature of 35°C, pH 6.5, 1.2 g L<sup>-1</sup> of NH<sub>4</sub>Cl (as a nitrogen source) and 10 g L<sup>-1</sup> of mannose (as carbon source). Besides, the strain PUNAJAN1 has also shown the efficient hydrogen production ability of 0.23 L-H<sub>2</sub>/g-COD<sub>removed</sub>, when POME was subjected as a carbon source. Further, hydrothermally prepared nickel (NiO NPs) and cobalt oxide nanoparticles (CoO NPs) were added to POME with the range of 0.25 to 3.0 mg L<sup>-1</sup> POME. Results demonstrated 1.51 and 1.67 folds of noticeable enhancement in biohydrogen production from POME supplemented with 1.5 mg L<sup>-1</sup> NiO NPs and 1.0 mg L<sup>-1</sup> CoO NPs respectively, in comparison to the control. Furthermore, a statistical approach to optimize the production of photo-fermentative H<sub>2</sub> from dark fermented POME using Box–Behnken response surface methodology. Experimental data has shown a positive correlation between interdependence among various parameters (such as dilution of DPOME, initial pH and agitation regime) with improved photo-H<sub>2</sub> production, as significant enhancement of hydrogen yield from 0.79 to 3.11 mol-H<sub>2</sub>/mol-acetate was observed under the optimal condition of 40% of dilution of DPOME; pH 6.0; and agitation rate of 140 rev/min. The observed enhancement in photohydrogen production from DPOME under optimized conditions was almost fivefold. Finally, feasibility of TSDPF system in enhancing photo-H<sub>2</sub> production using POME has been successfully validated, where first stage fermentation was carried out using PUNAJAN1 strain (resulted 41% of COD<sub>removal</sub> along with hydrogen yield of 37.11 ml H<sub>2</sub>/g-COD) followed by second stage fermentation using 40% diluted DPOME with sterilized tap water (photo-fermentation). Applicability of using TSDPF system in increasing hydrogen yield (from 37.11 to 130.89 ml H<sub>2</sub>/g-COD) and COD<sub>removal</sub> rate (from 41 to 93%) has been implicated in this study which is reportedly far superior to single stage dark fermentation of POME. So, these results confirmed an effectual utilization of sequential dark-photo fermentation using dark POME can result in substantial hydrogen production and COD<sub>removal</sub>.



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## LIST OF SYMBOLS

$H_{(Ac)}$	ACETIC ACID
$H_{(Bu)}$	Butyric acid
$H_{(Pr)}$	Propionic acid
$FeCl_2$	Ferric chloride
$MgSO_4$	Magnesium sulphate
$NaOH$	Sodium hydroxide
w/v	Weight to volume
$m^3$	Meter cube
min	Minute
g/L or $gL^{-1}$	Gram per litre
M	Moles
nm	Nanometre
KJ	Kilo-joule
MJ	Mega joule
$H_2$	Hydrogen
L- $H_2$ /g-	Litre hydrogen per gram of cod removal
g-CODL-1	Gram COD per litre
CFU/ml	Colony forming unit per millilitre
$W/m^2$	Watt per meter square
EtOH	Ethanol
$^{\circ}C$	Degree Celsius
$COD_{removed}$	COD removal from substrate

## LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
BLAST	Basic local alignment search tool
BOD	Biochemical oxygen demand
CCD	Central composite design
COD	Chemical oxygen demand
CSTR	Continuous stirrer tank reactor
DNA	Deoxyribonucleic acid
DPOME	Dark fermented palm oil mill effluent
DO	Dissolved oxygen
DoE	Design of experiment
EIA	Energy information administration
H <sub>2</sub>	Hydrogen
HPP	Hydrogen production potential
HPR	Hydrogen production rate
HY	Hydrogen yield
N	Normality
NPs	Nano-particles
PCR	Polymerase chain reaction
POME	Palm oil mill effluent
RSM	Response surface methodology
RSM	Response surface methodology
SD	Standard deviation
TSS	Total suspended solids
UASB	Up-flow anaerobic sludge blanket
VFA	Volatile fatty acid
VSS	Volatile suspended solids

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